Effect of Evolocumab on Lipoprotein Particles

Peter P. Toth, MD, PhD,[a,b] Naveed Sattar, MD,[c] Dirk J. Blom, MD, PhD,[d] Seth S. Martin, MD, MHS,[b] Steven R. Jones, MD,[b] Maria Laura Monsalvo, MD,[e] Mary Elliott, MSci,[f] Mike Davis, MA,[e] Ransi Somaratne, MD,[e] David Preiss, PhD[g]

[a]CGH Medical Center, Sterling, IL, USA; [b]Ciccarone Center for the Prevention of Heart Disease, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [c]University of Glasgow, Glasgow, UK; [d]Division of Lipidology, Department of Medicine, University of Cape Town, Cape Town, South Africa; [e]Amgen Inc., Thousand Oaks, CA, USA; [f]Amgen Ltd., Cambridge, UK; [g]MRC Population Health Research Unit, Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK

Corresponding Author:

Peter P. Toth, MD, PhD

Director of Preventative Cardiology

CGH Medical Center

Sterling, Illinois

Email: Peter.Toth@cghmc.com

Tel: (815) 632-5093

FAX: (815) 626-5947

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ABSTRACT

The level of low-density lipoprotein cholesterol (LDL-C) reflects the cholesterol carried mainly by low-density lipoprotein particles (LDL-P). LDL-C, however, does not always correlate with LDL-P because of the variable amounts of cholesterol per particle. Consideration of LDL-P concentrations in addition to LDL-C may help guide therapeutic decisions in a select number of patients. Evolocumab is a fully human monoclonal antibody directed against proprotein convertase subtilisin-kexin type 9 that lowers both LDL-C and cardiovascular events. To evaluate the effect of evolocumab on serum levels and size of lipoprotein particles, we conducted a post-hoc subanalysis of 619 patients from the DESCARTES trial, a 52-week, randomized, double-blind, placebo-controlled, global study of patients with hyperlipidemia. At baseline, mean LDL-P concentration was 1,077 nmol/L for the placebo group and 1,100 nmol/L for the evolocumab group. In patients receiving evolocumab, week 52 total LDL-P concentration decreased to 610 nmol/L, a treatment difference of 50% versus placebo. Evolocumab also reduced concentrations of medium VLDL-P, small VLDL-P, and IDL-P: median (Q1, Q3) changes were –15.2% (–48, 48), –29% (–54, 18), and –36% (–70, 22), respectively. Mean (95% CI) percent changes in total LDL particle size in the evolocumab group was –1.7 (–2.0, –1.4); percent changes in HDL and VLDL particle sizes were 1.1 (0.7, 1.5) and 8.7 (7.0, 10.5), respectively. Changes in total LDL, HDL, and VLDL particle sizes (versus placebo) were all significant ($P < .001$). In conclusion, evolocumab significantly lowers atherogenic lipoprotein particles including low-density and remnant lipoproteins.

Keywords (2-4): cholesterol, evolocumab, lipoprotein, remnant lipoproteins

INTRODUCTION

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease that inhibits LDL receptor (LDL-R) recycling. Evolocumab is a fully human monoclonal antibody that targets PCSK9 and prevents it from binding to the LDL-R. This increases LDL-R density on the surface of hepatocytes and, consequently, clearance rates of LDL-C from the blood. Evolocumab markedly lowers LDL-C in multiple clinical settings[1-6] and reduces the risk of cardiovascular events.[7] Although the effect of evolocumab on LDL-C levels is well characterized, little is known about its effects on lipoprotein particles or particle subfractions. The objective of this post hoc analysis was to evaluate the effect of evolocumab on lipoprotein particle concentrations as assessed by NMR spectroscopy in patients from the phase 3, 52-week DESCARTES (Durable Effect of PCSK9 Antibody Compared with Placebo Study, NCT01516879) trial.

METHODS

The DESCARTES trial was a 52-week, randomized, double-blind, placebo-controlled, multicenter, global study of patients with hyperlipidemia. Full details have been published previously.[1] DESCARTES was an LDL-C lowering trial and particle analyses were conducted post hoc. Briefly, patients were eligible to participate if they were aged 18 to 75 years with an LDL-C ≥ 1.9 mmol/L (75 mg/dL) and a fasting triglyceride level ≤ 4.5 mmol/L (400 mg/dL) following optimization of lipid lowering therapy in the run-in period. Based on cardiovascular risk, statin use, and baseline LDL-C, patients were assigned to diet alone, diet with 10 mg of atorvastatin daily, diet with 80 mg of atorvastatin daily, or diet with 80 mg of atorvastatin plus 10 mg of ezetimibe daily. The intensity of lipid-lowering therapy was increased stepwise until patients were at LDL-C target (NCEP ATP III, based on CV risk) or were receiving maximal therapy. After the run-in period, eligible patients were randomized 1:2 to receive placebo or evolocumab 420 mg monthly. For this subanalysis, patients were randomly selected with a greater weight given to patients with diabetes and patients who received placebo (to produce a

ratio nearer to 1:1 between treatment groups). Baseline was defined as study day 1 following lipid stabilization. Lipids and lipoprotein particle concentrations were analyzed at day 1 following lipid stabilization (baseline) and week 52. An independent ethics committee/institutional review board approved the protocol prior to study initiation at all sites. All patients provided written informed consent before enrollment.

Serum concentrations levels of lipoprotein particles (LDL, high-density lipoprotein [HDL], and VLDL/chylomicrons) and remnant lipoproteins (including small VLDL and IDL), as well as particle sizes, were measured using nuclear magnetic resonance (NMR) spectroscopy.[8] This method uses the amplitude of terminal lipid methyl group NMR signals to determine the particle concentration of lipoprotein subclasses.[8] Signal amplitudes were deconvoluted using the LipoProfile-3 algorithm, a model that is able to differentiate > 30 discrete subpopulations of lipoprotein particles.[8] Briefly, diameter ranges of lipoprotein subclasses are: VLDL, 27 - >60 nm; IDL, 23 – 27 nm; LDL, 18 – 23 nm; and HDL, 7.3 – 13 nm.[8,9] LDL-C was calculated using the Friedewald equation; VLDL-C was determined by ultracentrifugation. Baseline lipid levels (LDL-C, HDL-C, triglycerides, VLDL-C, and total cholesterol) were calculated by taking the mean of screening and day 1 values if available. IDL-C levels were not measured in the DESCARTES study.

LDL-P, and HDL-P were analyzed using mean absolute values at week 52 and mean percent change from baseline to week 52. The treatment differences for evolocumab versus placebo for these analytes were tested using two-sample t-tests. Large LDL-P, small LDL-P, medium VLDL-P, small VLDL-P, VLDL-P/chylomicron, and IDL-P were analyzed using median absolute values at week 52 and median percent change from baseline to week 52. The treatment differences for evolocumab versus placebo for these analytes were tested using Wilcoxon rank sum tests. All p-values are nominal, and no adjustment for multiplicity was performed. Particle sizes were determined using the total weighted average size for all subclasses of VLDL, LDL, and HDL particles. Differences were tested using two-sample t-tests.

No imputation was performed for missing data. Statistical analyses were conducted in SAS version 9.4 (SAS Institute, Cary, NC).

We evaluated the effect of evolocumab on LDL-P under conditions of baseline concordance and discordance (LDL-C > LDL-P or LDL-C < LDL-P). Discordance was defined as LDL-C and LDL-P values differing by > 14 percentile units resulting in approximately equal numbers of patients categorized as either concordant or discordant.[9]

RESULTS

Demographic data, baseline characteristics, lipid levels, and lipoprotein and remnant particle concentrations were available in 619 patients (Table 1). In total, 55% and 49% were women; 83% and 78% were white; and 14% and 18% had coronary artery disease, for placebo and evolocumab cohorts, respectively. This population is enriched for patients with diabetes, who made up 15% and 23% of the placebo and evolocumab groups.

At baseline, lipid measures and lipid particle values were comparable for placebo and evolocumab groups (Table 1). Briefly, for the placebo and evolocumab groups, the mean ± SD LDL-C was 99.8 mg/dL ± 21.6 and 100.7 mg/dL ± 23.8, respectively; the mean ± SD LDL-P was 1077.1 nmol/L ± 274.5 and 1100.2 nmol/L ± 286.0, respectively. The remainder of the lipoprotein values can be found in Table 1.

Beneficial changes were seen in LDL-C and other lipids in patients treated for 52 weeks with evolocumab (Table 2). At week 52, average LDL-C was markedly reduced, HDL-C increased, and triglycerides reduced in the evolocumab group compared with placebo (Table 2). Mean ± SE percent changes in LDL-C from baseline to week 52 for the placebo and evolocumab groups were 7% ± 1.8 and –53% ± 1.7, respectively. Evolocumab reduced apolipoprotein B (apoB), reduced the apoB/A1 ratio, and increased apoAI. (Table 2).

Changes in lipoprotein particle concentrations are reported in Table 3. At week 52, very low levels of total LDL-P were achieved in the majority of patients taking evolocumab.

Compared with placebo, evolocumab induced reductions in total LDL-P as well as small and large LDL-P. Evolocumab increased total HDL-P, as well as its small, medium, and large HDL-P species. 52 weeks evolocumab therapy was associated with reductions in the median total VLDL-P/chylomicron, medium VLDL-P, small VLDL-P, and IDL-P. The concentration of large VLDLwas increased by evolocumab..

Lipoprotein particle changes were also analyzed according to glycemic status at baseline, with patients differentiated by whether they had type 2 diabetes, impaired fasting glucose or met the defined diagnostic criteria for metabolic syndrome (modified from AHA/NHLBI).[10] There was remarkable consistency in the effect of evolocumab on serum levels of LDL-P, HDL-P, VLDL-P/chylomicrons, and IDL-P, with no substantive differences between these patient groups (Supplemental Table 1).

Distribution of LDL-C and LDL-P values at baseline are shown in Figure 1. Compared with placebo, evolocumab induced robust reductions in both LDL-C and total LDL-P, regardless of their concordance status at baseline (Table 4). These results were comparable to those obtained using an alternative method of estimating LDL-C (Supplemental Table 2).

The net size of LDL-P decreased significantly for the group as a whole, as well as for patients with impaired fasting glucose or metabolic syndrome; a decrease (not significant) was also observed for those with type 2 diabetes (Table 5). Evolocumab increased HDL particle and VLDL particle sizes for all groups.

DISCUSSION

This post hoc analysis of patients enrolled in the DESCARTES trial demonstrates that evolocumab induces marked reductions in LDL-C and LDL-P after 52 weeks of evolocumab exposure. Prior to the DESCARTES analysis, the effect of evolocumab on lipoprotein particles had only been assessed at two weeks after initiating evolocumab. Here, favorable changes of lipoprotein particle concentration and size are observed shortly after initiating evolocumab and

are maintained with long-term treatment. Furthermore, these patterns are consistent across this study population which was enriched for patients with diabetes. Both LDL-C and LDL-P were reduced from the Framingham 20th percentile to < 2nd percentile, which represents a marked shift in atherogenic lipoprotein burden. Put in context, in a comprehensive analysis of 8 studies, the mean percent reduction of LDL-P is 31 and the mean attained LDL-P percentile is 51 with statin monotherapy.[11] As demonstrated herein, the adjuvant use of evolocumab in patients treated with a statin provides more substantial reductions in these two measures. Evolocumab substantially reduced triglyceride enriched lipoprotein particles including a sum of chylomicron and large VLDL particles, small VLDL-P, and IDL-P. Both HDL-C and HDL-P increased. This therapeutic impact on lipoprotein fractions of evolocumab was consistent across all patient groups studied.

Evolocumab reduced both large and small LDL-P. The percentage reduction in large LDL-P was approximately twice that of small LDL-P, which likely accounts for the reduction in average LDL particle size. This may be a manifestation of less efficient clearance of small, dense LDL particles by the LDL-R compared to larger LDL particles.[12] Medium and large HDL-P increased and this was associated with a modest rise in average HDL particle size. Although small VLDL-P decreased, there was a rise in large VLDL-P. The precise etiology for this increase is not clear.

Overall, the changes reported here are consistent with those reported with alirocumab (150 mg every two weeks), although that study was shorter in duration (12 weeks), excluded patients with diabetes, and involved a smaller sample size of 31 patients.[13]

The effect of evolocumab on lipoprotein particle number and size were consistent regardless of concordance at baseline and regardless of whether the patient had diabetes, impaired fasting glucose, or metabolic syndrome. If LDL-C and LDL-P values are discordant, LDL-P levels may be important to consider when assessing cardiovascular risk. This could be important in patients with diabetes, who may be more likely to have discordance.[9] Thus, there

may be clinical importance in quantifying the effects of any lipid-lowering therapy on LDL-P as well as LDL-C.

The monoclonal antibodies directed against PCSK9 can reduce LDL-C to very low levels, especially when used in combination with high-dose, high-potency statins. Though there have been reservations about the safety of very low LDL-C levels, no safety signals have been discerned in either the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT),[14,15] in which the mean LDL-C level at 1 year was 53.2 mg per deciliter in the simvastatin plus ezetimibe group, or the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial, in which 42% of patients in the evolocumab group achieved an LDL-C level of 25 mg per deciliter or lower at 48 weeks.[7] As shown here, even when LDL-C approaches very low levels, there are still a substantial number of LDL particles available in serum irrespective of concordance.

Evolocumab provides robust reductions in LDL-C and LDL-P secondary to its capacity to significantly upregulate expression of the LDL receptor. However, PCSK9 also regulates expression of the VLDL receptor, LDL receptor-related protein, and apolipoprotein E receptor 2 (apoER2).[16,17] In this study evolocumab therapy clearly potentiated reductions of triglyceride-enriched lipoproteins, including VLDL-P and IDL-P. It is possible that the aforementioned receptors may play roles in facilitating the clearance of these lipoproteins thereby leading to their augmented clearance from the systemic circulation. The LDLR is also involved in remnant clearance via apoE binding and upregulating it may thus also improve remnant clearance.[18]

Though the mechanism is not yet well understood, evolocumab treatment is associated with modest elevations in both HDL-C and HDL-P. To date, there is no evidence to suggest that PCSK9 modulates HDL-P production or clearance; thus, the effect of evolocumab on HDL levels may be largely indirect. With lower availability of apoprotein B-containing particles in serum (LDL, VLDL, and IDL), it is possible that there is less enrichment of the HDL fraction with

triglyceride by cholesterol ester transfer protein. This could help to preserve or increase levels of HDL in blood via less catabolism of HDL by hepatic lipase.

Though the small intestine does express PCSK9,[19] it is not yet clear whether PCSK9 plays any role in regulating chylomicron production or secretion. Current NMR methodology and deconvolution programs cannot independently estimate serum levels of chylomicrons. Among patients treated with evolocumab in DESCARTES, the combined fraction of VLDL/chylomicrons (lipoproteins secreted from the liver and jejunum) was decreased. Considering that all patients were fasting and patients with hypertriglyceridemia (< 4.5 mmol/L) were excluded from DESCARTES, it is unlikely that this subset of patients would have significant amounts of circulating chylomicrons. Whether the VLDL/chylomicron reduction was secondary to increased clearance, reduced production, or both, and from which fractions remains to be determined.

In conclusion, evolocumab significantly lowered atherogenic lipoprotein particles including low-density lipoprotein and remnant lipoproteins.

DISCLOSURES

PPT reports receiving consulting fees from Amarin, Amgen, AstraZeneca, Gemphire, Kowa, Merck, and Sanofi/Regeneron and serving on speakers bureaus for Amarin, Amgen, Kowa, Merck, and Regeneron-Sanofi. **NS** reports receiving consulting fees from Amgen, Sanofi, and Merck. **DJB** reports that his institution has received research grants from Sanofi-Aventis, Regeneron, Novartis, Eli Lilly & Company, Amgen, and Aegerion; and reports receiving consulting fees from Aegerion and Gemphire for serving on steering committees; receiving fees for serving on advisory boards for Sanofi-Aventis, Aegerion, Amgen, AstraZeneca, and MSD; receiving honoraria for lectures from Sanofi-Aventis, Regeneron, Aegerion, Amgen, AstraZeneca, MSD, Pfizer, Servier, and Unilever; receiving travel assistance from Amgen and Aegerion; and receiving non-financial support (editorial assistance and statistical analyzes) from Sanofi-Aventis and Regeneron. **SSM** reports research grants to his institution from the Aetna Foundation, American Heart Association, Maryland Innovation Initiative, Nokia/Withings, Apple, and Google; and he reports receiving consulting fees from Abbott Nutrition, Pressed Juicery, Quest Diagnostics, Amgen, Sanofi/Regeneron, and the Pew Research Center. **SSM** and **SRJ** reports serving as co-inventors on a pending patent for a novel method of LDL-C calculation filed by Johns Hopkins University. **SRJ** has no other conflicts of interest to report. **MLM, ME, RS,** and **MD** are all Amgen employees and report that they own Amgen stocks/stock options. **DP** reports having received consulting fees from Sanofi on three occasions, all in 2013/2014, during previous employment.

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ROLE OF THE FUNDER/SPONSOR

Amgen had a role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, and approval of the manuscript.

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TABLES

Table 1. Baseline demographics, clinical characteristics, lipids, and lipoprotein particles

Variable	Placebo (n = 283)	Evolocumab 420 mg QM (n = 336)
Women	157 (55.5%)	166 (49.4%)
White	234 (82.7%)	263 (78.3%)
Age (years), mean ± SD	57.1 ± 10.0	56.4 ± 10.6
Coronary artery disease	41 (14.5%)	59 (17.6%)
Cerebrovascular or peripheral artery disease	11 (3.9%)	17 (5.1%)
Type 2 diabetes	43 (15.2%)	77 (22.9%)
Body mass index (kg/m^2), mean ± SD	30.5 ± 5.7	30.2 ± 6.2
Waist circumference (cm), mean ± SD	100.9 ± 14.3	99.9 ± 13.5
LDL-C (mg/dL), mean ± SD	99.8 ± 21.6	100.7 ± 23.8
VLDL-C (mg/dL), median (Q1, Q3)	18.5 (13.0, 28.5)	18.5 (13.0, 25.0)
HDL-C (mg/dL), mean ± SD	53.4 ± 16.4	51.7 ± 15.0
Triglycerides (mg/dL), median (Q1, Q3)	112.0 (86.0, 162.0)	110.8 (79.5, 149.5)
Total cholesterol (mg/dL), mean ± SD	179.1 ± 27.6	176.9 ± 28.3
LDL-P total (nmol/L), mean ± SD	1077.1 ± 274.5	1100.2 ± 286.0
Large LDL-P (nmol/L), median (Q1, Q3)	343.0 (214.0, 466.0)	359.0 (253.0, 490.0)
Small LDL-P (nmol/L), median (Q1, Q3)	614.0 (463.0, 765.0)	613.0 (463.0, 775.0)
HDL-P total (μmol/L), mean ± SD	35.5 ± 6.4	34.6 ± 6.0
Large HDL-P (μmol/L), median (Q1, Q3)	5.0 (3.3, 7.6)	4.6 (2.9, 7.3)
Medium HDL-P (μmol/L), median (Q1, Q3)	9.4 (6.1, 13.0)	9.3 (6.3, 12.9)
Small HDL-P (μmol/L), median (Q1, Q3)	20.1 (16.4, 23.1)	19.3 (16.0, 22.6)
VLDL-P/Chylomicron total (nmol/L), median (Q1, Q3)	50.4 (34.1, 71.5)	43.9 (28.5, 69.7)
Large VLDL-P/Chylomicron (nmol/L), median (Q1, Q3)	3.3 (1.6, 6.7)	2.9 (1.2, 5.7)
Medium VLDL-P (nmol/L), median (Q1, Q3)	17.5 (10.0, 31.0)	16.2 (9.3, 27.6)
Small VLDL-P (nmol/L), median (Q1, Q3)	26.1 (17.7, 38.3)	23.2 (15.3, 35.2)
IDL-P (nmol/L), median (Q1, Q3)	76.0 (40.0, 124.0)	72.0 (40.0, 115.0)

HDL-C, high-density lipoprotein cholesterol; IDL-P, intermediate-density lipoprotein particle concentration; LDL-C, low-density lipoprotein cholesterol; Q1, Q3, first and third quartiles; SD, standard deviation; VLDL-C, very low-density lipoprotein cholesterol; VLDL-P, very low-density lipoprotein particle concentration

*Defined as having been previously diagnosed with diabetes, baseline use of glucose-lowering medication, baseline fasting plasma glucose ≥ 126 mg/dL, or baseline HbA1c ≥ 6.5%

Table 2. Percent Change in Lipids and Apolipoproteins from Baseline to Week 52

Variable	Placebo		Evolocumab 420 mg QM	
	n	Mean ± SE or Median (Q1, Q3)	n	Mean ± SE or Median (Q1, Q3)
LDL-C	250	7.0 ±1.8	309	–53.0 ±1.7
HDL-C	255	0.3 ± 0.9	313	5.7 ± 0.9
VLDL-C	253	9.1 (–19.1, 48.5)	311	–13.0 (–37.5, 20.0)
ApoB	267	2.2 ± 1.4	325	–42.6 ± 1.2
ApoA1	267	–1.2 ± 0.7	325	2.5 ± 0.6
ApoB/ApoA1	267	4.1 ± 1.5	325	–43.2 ± 1.4
Triglycerides	255	3.5 (–16.7, 24.8)	313	–9.5 (–26.5, 12.4)
Total cholesterol	255	4.7 ± 1.2	313	–29.3 ± 1.0

ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Q1, Q3, first and third quartiles; SE, standard error; VLDL-C, very low-density lipoprotein cholesterol

Table 3. Lipoprotein and Remnant Particle Concentrations and Percent Change from Baseline to Week 52

Variable	Placebo n		Evolocumab 420 mg QM n	
LDL-P total				
Mean ± SD, (nmol/L)	246	1110.3 ± 326.2	300	609.8 ± 336.9
Percent change from baseline, mean [95% CI]	236	6.4 [2.9, 9.9]	294	–44.1[a] [–47.2, –40.9]
HDL-P total				
Mean ± SD, (μmol/L)	246	35.4 ± 6.1	300	37.5 ± 6.2
Percent change from baseline, mean [95% CI]	236	–0.1 [–1.6, 1.4]	294	9.4[a] [7.5, 11.4]
Large LDL-P				
Median (Q1, Q3), (nmol/L)	246	362.5 (231.0, 532.0)	300	91.5 (33.0, 180.5)
Percent change from baseline, median (Q1, Q3)	233	5.1 (–22.8, 43.4)	292	–73.7[a] (–89.8, –50.9)
Small LDL-P				
Median (Q1, Q3), (nmol/L)	246	615.0 (460.0, 775.0)	300	367.0 (274.0, 507.5)
Percent change from baseline, median (Q1, Q3)	236	3.8 (–16.9, 29.0)	294	–35.4[a] (–56.7, –11.4)
VLDL-P and Chylomicron total				
Median (Q1, Q3), (nmol/L)	246	49.4 (32.3, 75.4)	300	35.8 (25.1, 53.7)
Percent change from baseline, median (Q1, Q3)	236	–0.3 (–26.3, 31.7)	294	–15.3[b] (–39.3, 15.4)
Large VLDL-P and Chylomicron				
Median (Q1, Q3), (nmol/L)	246	3.1 (1.4, 6.0)	300	3.1 (1.6, 6.4)
Percent change from baseline, median (Q1, Q3)	236	1.0 (–41.8, 60.4)	294	10.5 (–26.1, 100.0)
Medium VLDL-P				
Median (Q1, Q3), (nmol/L)	246	18.2 (10.3, 31.0)	300	15.1 (8.2, 25.3)
Percent change from baseline, median (Q1, Q3)	235	7.1 (–36.3, 50.8)	292	–15.2 (–47.7, 48.3)
Small VLDL-P				

Median (Q1, Q3), (nmol/L)	246	26.2 (16.2, 37.0)	300	16.8 (10.8, 25.1)
Percent change from baseline, median (Q1, Q3)	236	–7.5 (–33.1, 30.4)	293	–29.0[a] (–54.1, 18.3)
IDL-P				
Median (Q1, Q3), (nmol/L)	246	74.0 (44.0, 125.0)	300	45.5 (26.0, 79.0)
Percent change from baseline, median (Q1, Q3)	236	0 (–47.4, 87.5)	294	–36.2[a] (–69.8, 22.0)

HDL-C, high-density lipoprotein cholesterol; IDL-P, intermediate-density lipoprotein particle concentration; LDL, low-density lipoprotein; LDL-P, LDL particle concentration; Q1, Q3, first and third quartiles; QM, once every month; SD, standard deviation; VLDL-P, very low-density lipoprotein particle concentration

P values reported are for treatment differences (evolocumab versus placebo) tested using two-sample t-test for LDL-P and HDL-P. All other parameters were analyzed using the Wilcoxon rank sum test.

[a]$P < .0001$; [b]$P < .001$; [c]$P < .01$

Table 4. Percent Change in LDL-P and LDL-C from Baseline to Week 52 by Concordant/Discordant Status

	Concordant (n = 272)		Discordant LDL-P < LDL-C (n = 128)		Discordant LDL-P > LDL-C (n = 130)	
	Placebo (n = 123)	Evolocumab (n = 149)	Placebo (n = 58)	Evolocumab (n = 70)	Placebo (n = 55)	Evolocumab (n = 75)
LDL-P, mean ± SE	6.2 ± 2.7	–47.4 ± 1.9*	9.4 ± 3.2	–37.5 ± 3.8*	3.6 ± 3.4	–43.6 ± 3.4*
LDL-C, mean ± SE	7.6 ± 2.9	–55.4 ± 2.0*	3.5 ± 2.6	–50.7 ± 3.9*	13.7 ± 3.7	–48.5 ± 4.4*

LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle concentration; SE, standard error
P values reported are for treatment differences (evolocumab versus placebo) tested using two-sample t-test
*$P < .0001$

Table 5. Percent Change in Particle Sizes from Baseline to Week 52

	Placebo		Evolocumab 420 mg QM	
Variable	**n**	**Mean (95% CI)**	**n**	**Mean (95% CI)**
LDL				
All	234	0 (–0.3, 0.2)	244	–1.7 (–2.0, –1.4)[a]
Type 2 diabetes*	33	–0.2 (–1.0, 0.6)	46	–0.8 (–1.6, –0.1)
Impaired fasting glucose†	83	–0.2 (–0.6, 0.2)	85	–2.0 (–2.6, –1.5)[a]
Metabolic syndrome‡	83	–0.1 (–0.5, 0.3)	81	–1.6 (–2.1, –1.1)[a]
HDL				
All	236	–0.02 (–0.4, 0.4)	294	1.1 (0.7, 1.5)[b]
Type 2 diabetes*	33	–0.3 (–1.8, 1.1)	57	1.1 (0.3, 1.9)
Impaired fasting glucose†	84	0.1 (–0.6, 0.8)	103	1.1 (0.5, 1.7)
Metabolic syndrome‡	84	0.3 (–0.3, 1.0)	94	1.4 (0.5, 2.2)
VLDL				
All	234	0.9 (–0.8, 2.5)	287	8.7 (7.0, 10.5)[a]
Type 2 diabetes*	31	4.0 (–0.9, 8.8)	55	9.2 (5.0, 13.5)
Impaired fasting glucose†	84	1.2 (–1.6, 4.0)	102	8.2 (5.6, 10.9)[b]
Metabolic syndrome‡	84	1.3 (–1.3, 3.9)	93	6.8 (4.4, 9.3)[c]

CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein

[a]$P < .0001$; [b]$P < .001$; [c]$P < .01$

*Defined as having been previously diagnosed with diabetes, baseline use of glucose-lowering medication, baseline FPG ≥ 126 mg/dL, or baseline HbA1c ≥ 6.5%

†Defined as the absence of type 2 diabetes with a fasting plasma glucose ≥ 100 mg/dL and < 126 mg/dL at baseline

‡Defined using modified AHA/NHLBI criteria as the absence of type 2 diabetes and the presence of 3 or more of the following components: elevated waist circumference (non-Asian men ≥ 102 cm, non-Asian women ≥ 88 cm, Asian men ≥ 90 cm, Asian women ≥ 80 cm); triglycerides ≥ 1.7 mmol/L (150 mg/dL); low HDL cholesterol (men < 40 mg/dL, women < 50 mg/dL); high blood pressure (systolic ≥ 130 mmHg, diastolic ≥ 85 mmHg, medical history of hypertension); and fasting plasma glucose ≥ 100 mg/dL

Supplemental Table 1. Lipoprotein and Remnant Particle Mean Concentrations and Percent Change from Baseline to Week 52 in Patients with Dysglycemia or Metabolic Syndrome

	Placebo		Evolocumab 420 mg QM	
	n		n	
LDL-P total				
Mean (SD), nmol/L				
All patients	246	1110.3 (326.2)	300	609.8 (336.9)
Type 2 diabetes*	35	1095.6 (332.6)	58	653.4 (379.7)
Impaired fasting glucose†	88	1098.1 (329.2)	104	585.5 (317.4)
Metabolic syndrome‡	89	1156.8 (341.7)	95	662.2 (336.6)
Percent change from baseline, mean [95% CI]				
All patients	236	6.4 [2.9, 9.9]	294	–44.1[a] [–47.2, –40.9]
Type 2 diabetes*	33	5.7 [–2.5, 13.8]	57	–42.4[a] [–49.8, –35.0]
Impaired fasting glucose†	84	4.9 [–0.9, 10.6]	103	–46.3[a] [–51.2, –41.3]
Metabolic syndrome‡	84	3.6 [–1.7, 9.0]	94	–43.6[a] [–48.7, –38.6]
HDL-P total				
Mean (SD), □mol/L				
All patients	246	35.4 (6.1)	300	37.5 (6.2)
Type 2 diabetes*	35	32.4 (6.1)	58	36.4 (6.9)
Impaired fasting glucose†	88	35.6 (6.2)	104	38.5 (5.8)
Metabolic syndrome‡	89	34.6 (6.9)	95	36.6 (6.6)
Percent change from baseline, mean [95% CI]				
All Patients	236	–0.1 [–1.6, 1.4]	294	9.4[a] [7.5, 11.4]
Type 2 diabetes*	33	–1.1 [–5.0, 2.8]	57	7.3[c] [4.0, 10.7]
Impaired fasting glucose†	84	0.7 [–2.3, 3.7]	103	10.5[b] [6.4, 14.5]
Metabolic syndrome‡	84	0.0 [–2.9, 2.9]	94	10.1[b] [5.5, 14.8]
Large LDL-P				
Median (Q1, Q3), nmol/L				
All patients	246	362.5 (231.0, 532.0)	300	91.5 (33.0, 180.5)
Type 2 diabetes*	35	313.0 (191.0, 451.0)	58	88.0 (38.0, 197.0)
Impaired fasting glucose†	88	322.0 (194.0, 481.0)	104	83.0 (24.5, 153.5)
Metabolic syndrome‡	89	273.0 (159.0, 414.0)	95	97.0 (31.0, 169.0)
Percent change from baseline, median (Q1, Q3)				

All patients	233	5.1 (–22.8, 43.4)	292	–73.7[a] (–89.8, –50.9)
Type 2 diabetes*	33	–3.1 (–28.0, 45.7)	56	–65.8[a] (–85.9, –47.5)
Impaired fasting glucose[†]	83	3.0 (–23.3, 38.6)	103	–77.8[a] (–92.1, –48.8)
Metabolic syndrome[‡]	81	3.0 (–30.5, 45.3)	93	–67.3[a] (–88.7, –45.6)
Small LDL-P				
Median (Q1, Q3), nmol/L				
All patients	246	615.0 (460.0, 775.0)	300	367.0 (274.0, 507.5)
Type 2 diabetes*	35	660.0 (522.0, 836.0)	58	437.0 (312.0, 496.0)
Impaired fasting glucose[†]	88	622.5 (473.0, 786.5)	104	360.5 (283.0, 502.0)
Metabolic syndrome[‡]	89	695.0 (589.0, 892.0)	95	454.0 (300.0, 610.0)
Percent change from baseline, median (Q1, Q3)				
All patients	236	3.8 (–16.9, 29.0)	294	–35.4[a] (–56.7, –11.4)
Type 2 diabetes*	33	6.0 (–8.1, 50.7)	57	–35.5[a] (–56.8, –21.8)
Impaired fasting glucose[†]	84	5.6 (–15.5, 29.0)	103	–35.2[a] (–56.7, –2.1)
Metabolic syndrome[‡]	84	4.0 (–11.5, 27.5)	94	–32.6[a] (–54.4, –12.6)
VLDL-P and Chylomicron total				
Median (Q1, Q3), nmol/L				
All patients	246	49.4 (32.3, 75.4)	300	35.8 (25.1, 53.7)
Type 2 diabetes*	35	43.8 (31.3, 58.1)	58	35.2 (27.0, 48.3)
Impaired fasting glucose[†]	88	51.3 (32.7, 77.9)	104	35.1 (25.0, 50.1)
Metabolic syndrome[‡]	89	67.7 (46.8, 86.0)	95	42.1 (26.3, 61.4)
Percent change from baseline, median (Q1, Q3)				
All patients	236	–0.3 (–26.3, 31.7)	294	–15.3[b] (–39.3, 15.4)
Type 2 diabetes*	33	–10.3 (–29.0, 14.6)	57	–17.3 (–39.3, 19.0)
Impaired fasting glucose[†]	84	–4.4 (–30.9, 29.9)	103	–12.9 (–41.3, 14.2)
Metabolic syndrome[‡]	84	–4.3 (–25.4, 16.2)	94	–24.5[b] (–43.7, –3.0)
Large VLDL-P and Chylomicron				
Median (Q1, Q3), nmol/L				
All patients	246	3.1 (1.4, 6.0)	300	3.1 (1.6, 6.4)
Type 2 diabetes*	35	3.1 (1.5, 4.6)	58	4.0 (2.1, 7.4)
Impaired fasting glucose[†]	88	4.0 (1.8, 7.1)	104	3.0 (1.7, 5.9)
Metabolic syndrome[‡]	89	5.6 (3.0, 9.6)	95	4.6 (2.2, 8.3)
Percent change from baseline, median (Q1, Q3)				

All patients	236	1.0 (–41.8, 60.4)	294	10.5 (–26.1, 100.0)
Type 2 diabetes*	33	26.7 (–23.8, 66.7)	57	10.9 (–33.3, 62.3)
Impaired fasting glucose†	84	2.8 (–43.6, 60.4)	103	0.0 (–25.0, 82.9)
Metabolic syndrome‡	84	–1.9 (–42.8, 60.4)	94	–3.3 (–31.3, 45.5)
Medium VLDL-P				
Median (Q1, Q3), nmol/L				
All patients	246	18.2 (10.3, 31.0)	300	15.1 (8.2, 25.3)
Type 2 diabetes*	35	16.6 (9.7, 22.7)	58	15.8 (8.2, 21.4)
Impaired fasting glucose†	88	19.1 (9.4, 31.1)	104	13.9 (8.5, 24.7)
Metabolic syndrome‡	89	25.4 (17.2, 37.0)	95	18.8 (9.0, 28.1)
Percent change from baseline, median (Q1, Q3)				
All patients	235	7.1 (–36.3, 50.8)	292	–15.2 (–47.7, 48.3)
Type 2 diabetes*	33	–10.2 (–49.9, 32.0)	57	–17.4 (–45.1, 38.5)
Impaired fasting glucose†	84	3.8 (–40.0, 40.4)	103	–14.1 (–48.4, 71.6)
Metabolic syndrome‡	84	–1.3 (–39.9, 32.8)	93	–21.3 (–47.9, 19.7)
Small VLDL-P				
Median (Q1, Q3), nmol/L				
All patients	246	26.2 (16.2, 37.0)	300	16.8 (10.8, 25.1)
Type 2 diabetes*	35	24.4 (14.6, 32.7)	58	18.5 (9.6, 25.0)
Impaired fasting glucose†	88	27.9 (15.7, 39.0)	104	16.2 (12.1, 23.7)
Metabolic syndrome‡	89	31.6 (19.0, 42.8)	95	17.0 (11.2, 27.1)
Percent change from baseline, median (Q1, Q3)				
All patients	236	–7.5 (–33.1, 30.4)	293	–29.0[a] (–54.1, 18.3)
Type 2 diabetes*	33	–3.9 (–26.9, 64.0)	57	–22.9 (–51.2, 18.8)
Impaired fasting glucose†	84	–11.4 (–37.5, 20.9)	103	–28.9 (–53.6, 13.0)
Metabolic syndrome‡	84	–7.4 (–34.1, 19.5)	94	–34.6[c] (–52.7, 10.0)
IDL-P				
Median (Q1, Q3), nmol/L				
All patients	246	74.0 (44.0, 125.0)	300	45.5 (26.0, 79.0)
Type 2 diabetes*	35	68.0 (27.0, 85.0)	58	41.5 (29.0, 78.0)
Impaired fasting glucose†	88	75.0 (43.0, 129.0)	104	43.0 (23.0, 88.5)
Metabolic syndrome‡	89	79.0 (42.0, 134.0)	95	39.0 (20.0, 79.0)
Percent change from baseline, median (Q1, Q3)				
All patients	236	0 (–47.4, 87.5)	294	–36.2[a] (–69.8, 22.0)
Type 2 diabetes*	33	0 (–36.0, 34.9)	57	–33.8 (–69.0, 38.1)
Impaired fasting glucose†	84	–11.8 (–53.9, 93.8)	103	–37.8[c] (–70.6, 14.2)
Metabolic syndrome‡	84	–12.7 (–59.6, 72.1)	94	–53.1[b] (–79.3, 12.5)

HDL-C, high-density lipoprotein cholesterol; IDL-P, intermediate-density lipoprotein particle concentration; LDL, low-density lipoprotein; LDL-P, LDL particle concentration; Q1, Q3, first and third quartiles; QM, once every month; SD, standard deviation; VLDL-P, very low-density lipoprotein particle concentration

P values reported are for treatment differences (evolocumab versus placebo) within each subgroup, tested using two-sample t-test for LDL-P and HDL-P. All other parameters were analyzed using the Wilcoxon rank sum test

[a]*P* < .0001; [b]*P* < .001; [c]*P* < .01

[*]Defined as having been previously diagnosed with diabetes, baseline use of glucose-lowering medication, baseline FPG ≥ 126 mg/dL, or baseline HbA1c ≥ 6.5%

[†]Defined as the absence of type 2 diabetes with a fasting plasma glucose ≥ 100 mg/dL and < 126 mg/dL at baseline

[‡]Defined using modified AHA/NHLBI criteria as the absence of type 2 diabetes and the presence of 3 or more of the following components: elevated waist circumference (non-Asian men ≥ 102 cm, non-Asian women ≥ 88 cm, Asian men ≥ 90 cm, Asian women ≥ 80 cm); triglycerides ≥ 1.7 mmol/L (150 mg/dL); low HDL cholesterol (men < 40 mg/dL, women < 50 mg/dL); high blood pressure (systolic ≥ 130 mmHg, diastolic ≥ 85 mmHg, medical history of hypertension); and fasting plasma glucose ≥ 100 mg/dL

Supplemental Table 2. Percent Change in LDL-P and LDL-C Levels from Baseline to Week 52 by Concordance/Discordance Status Using Two LDL-C Estimation Methods

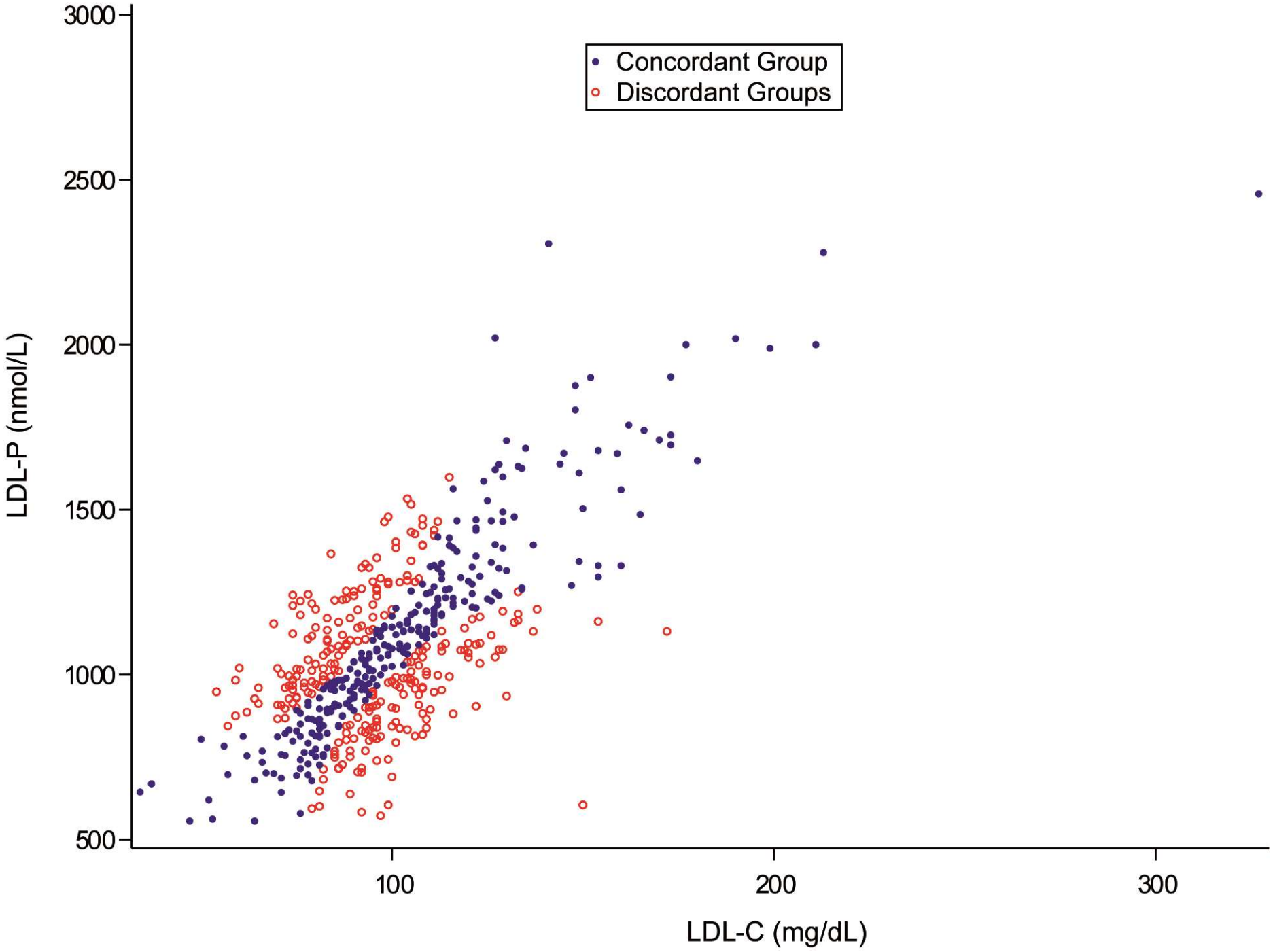
	Friedewald equation estimate						Triglycerides:VLDL-C ratio[1]					
	Concordant n = 272		Discordant LDL-P < LDL-C n = 128		Discordant LDL-P > LDL-C n = 130		Concordant n = 280		Discordant LDL-P < LDL-C n = 125		Discordant LDL-P > LDL-C n = 125	
	Pbo	Evo	Pbo	Evo	Pbo	Evo	Pbo	Evo	Pbo	Evo	Pbo	Evo
	n = 123	n = 149	n = 58	n = 70	n = 55	n = 75	n = 127	n = 153	n = 57	n = 68	n = 52	n = 73
LDL-P, Mean (SE)	6.2 (2.7)	–47.4 (1.9)	9.4 (3.2)	–37.5 (3.8)	3.6 (3.4)	–43.6 (3.4)	7.1 (2.6)	–45.4 (2.0)	7.8 (3.2)	–39.1 (3.7)	3.2 (3.6)	–46.0 (3.5)
LDL-C, Mean (SE)	7.6 (2.9)	–55.4 (2.0)	3.5 (2.6)	–50.7 (3.9)	13.7 (3.7)	–48.5 (4.4)	8.3 (2.7)	–51.5 (2.3)	1.3 (2.7)	–51.5 (3.1)	15.2 (3.8)	–47.8 (4.5)

CI, confidence interval; Evo, evolocumab; LDL-C, low-density lipoprotein cholesterol; P, particle concentration; Pbo, placebo; SE, standard error

[1]Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, Jones SR. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA* 2013;310:2061-8.

P values are for the evolocumab versus placebo comparison

**P* < .0001


Concordant Group
Discordant Groups
LDL-P (nmol/L)
3000
2500
2000
1500
1000
500
100
200
300
LDL-C (mg/dL)